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# Biological oscillations: Fluorescence monitoring by confocal microscopy

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ABSTRACT

Fluctuations play a vital role in biological systems. Single molecule spectroscopy has recently revealed many new kinds of fluctuations in biological molecules. In this account, we focus on structural fluctuations of an antigen-antibody complex, conformational dynamics of a DNA quadruplex, effects of taxol on dynamics of microtubules, intermittent red-ox oscillations at different organelles in a live cell (mitochondria, lipid droplets, endoplasmic reticulum and cell membrane) and stochastic resonance in gene silencing. We show that there are major differences in these dynamics between a cancer cell and the corresponding non-cancer cell.



## 1. Introduction

Many biological systems exhibit periodic, semi-periodic and intermittent oscillations [1–8]. Such bio-rhythms control a host of physiological functions ranging from cardiac signal [2], auditory perception in cray-fish and other animals [3] and enzyme reactions [4] to sustained glycolytic oscillation [5], gene expression [6], transcriptional regulation [7] and neuronal signal processing [8]. The origin of the oscillations includes structural oscillation and concentration oscillation of signalling molecules. Recent advances in single molecule spectroscopy have provided new ways to follow the fluctuations of molecules with unprecedented spatial resolution [9–18].

Flexibility and fluctuation is essential for biological function [15–18]. For instance, an enzyme-substrate (E-S) complex, with a lock-and-key fit, cannot afford to be rigid [15–18]. The enzyme must open up to allow the substrate to move to the active site and then, to hold them together for the reaction to occur and finally, to open up again to release the product. The structural fluctuation of an enzyme-substrate complex can be monitored by smFRET [19]. The efficiency of FRET ($\varepsilon_{FRET}$) is proportional to $R_{DA}^{-6}$ where $R_{DA}$ is the donor-acceptor distance [19]. In order to monitor fluctuations of the E-S complex, a donor (D) and an acceptor (A) are covalently attached to the enzyme and the substrate. Recent smFRET experiments, on such labelled enzyme-substrate complexes, have revealed a wide distribution of FRET efficiency [17,18]. This suggests that during the course of an enzymatic reaction the distance between the enzyme and the substrate fluctuates [15–18]. As a result, the rate constant of an enzymatic reaction may vary with time. These results in single molecule enzymology call for a serious re-consideration of the celebrated Michaelis-Menten model which assumes a unique rate constant [17].

A major source of structural fluctuation in a bio-molecule is conformational dynamics [20–28]. During conformational fluctuations, amino groups of certain residues/nucleo-bases may come in close proximity to a fluorophore covalently attached to a bio-molecule (e.g. protein). Subsequently, the amino group quenches fluorescence by electron transfer and gives rise to fluorescence intensity fluctuations. This forms the basis of photo-induced electron transfer fluorescence correlation spectroscopy (PET-FCS). This method has been applied to a wide variety of bio-molecule (DNA, proteins and receptors) [20–28].

For an environment sensitive fluorescent probe, emission maximum and quantum yield varies with polarity/viscosity of local environment [29]. Fluctuation in the local microenvironment of a bio-molecule may cause fluctuation in fluorescence intensity. In several studies, we used a thiol reactive dye (7-diethylamino-3-(4-maleimido-phenyl)-4-methylcoumarin), CPM. CPM is non-fluorescent in unbound state and is fluorescent only when bound to a thiol group (Scheme 1A and B). Thus making/breaking of the thiol-CPM bond may also be a source of fluctuation in fluorescence intensity of CPM.

In this account, we discuss five different kinds of structural fluctuations of biological systems. The first deals with dynamics of antigen-antibody complex. We demonstrate that this complex is not rigid and distance between the antigen and its specific antibody fluctuates over time. Second, we describe how formation and conformation dynamics of a DNA quadruplex may be monitored using FCS and smFRET. The third topic is polymerization and de-polymerization of microtubules which is crucial in cell division and growth. Fourth, many biological processes involve electron transfer and associated red-ox oscillations. We will

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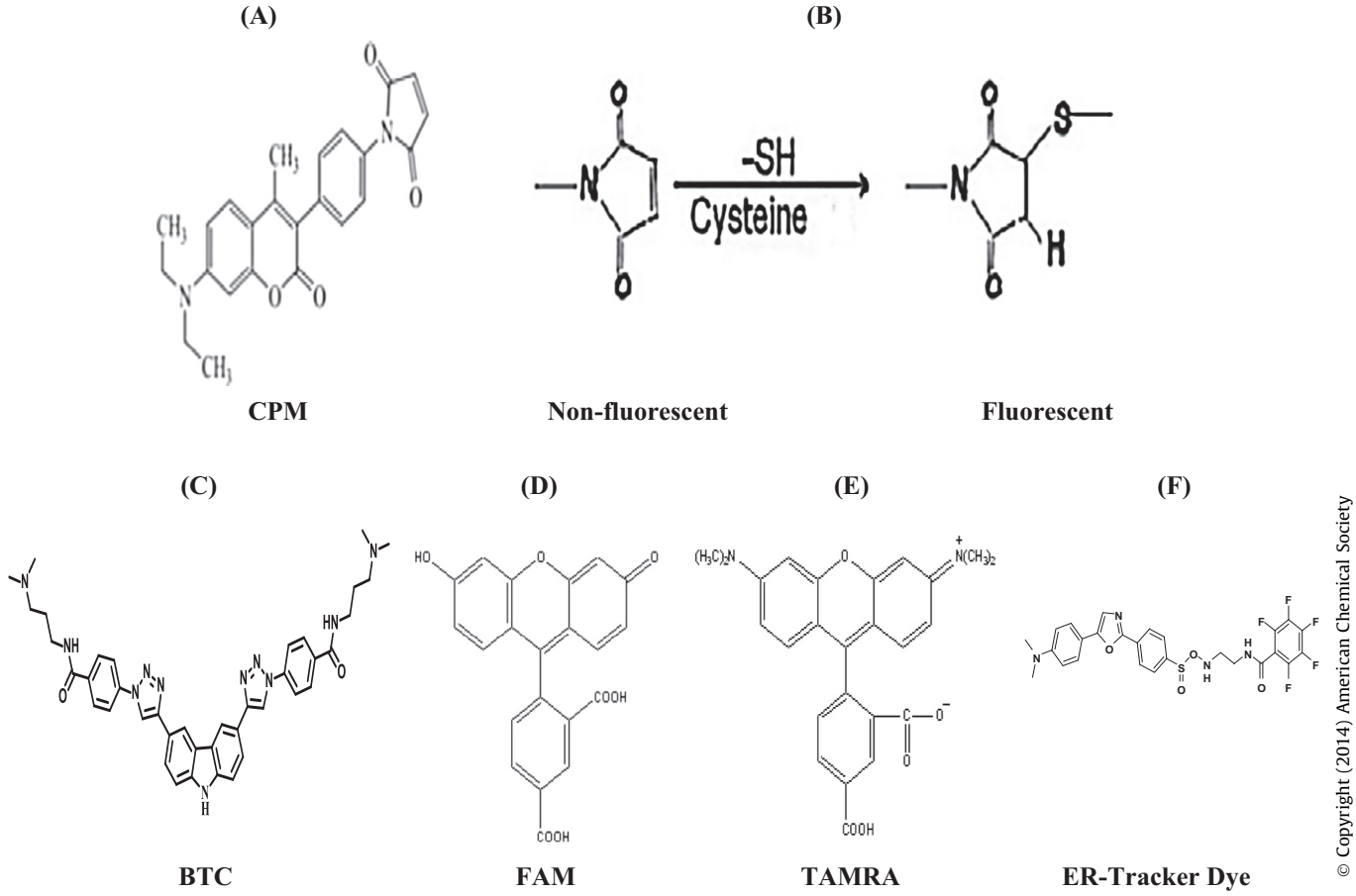


**Scheme 1.** Chemical structure of (A) CPM and (B) thiol labeling reaction by CPM resulting in transition from non-fluorescent to fluorescent state (reprinted with permission from Ref. [48]). Structure of (C) BTC, (D) FAM, (E) TAMRA and (F) ER Tracker dye.

illustrate this by studying oscillations at different organelles in a cell. The fifth and last issue is stochastic resonance in gene silencing in a cancer cell by short interfering RNA (siRNA). We show that the gene silencing in a cancer cell exhibit stochastic resonance. Further, we highlight many differences between a cancer cell and corresponding non-cancer cell in terms of biological oscillations and in drug delivery.

## 2. Structural fluctuation of antigen-antibody complex

An antigen binds to an antibody, in a highly specific manner. Very recently, we have studied structure and dynamics of an antigen-antibody complex by FCS and smFRET [30]. In our study, the antigen is enhanced GFP (EGFP) and the antibody is anti-EGFP $V_HH$-$His_6$. For smFRET experiments, the antigen (EGFP) itself acts as a donor. The acceptor is alexa 594 which is covalently attached to anti-EGFP $V_HH$-$His_6$. FCS study indicates that the structure of the antibody is quiet flexible with a time constant of conformational relaxation of 83 μs. Interestingly, when the antigen binds to the antibody, the conformational dynamics becomes nearly 3-fold slower (240 μs). This indicates that binding of antigen to the antibody makes the antigen-antibody complex less flexible [30].

In order to monitor the internal structural fluctuation of the antigen-antibody complex, we monitored real time fluctuation in efficiency of FRET ($\varepsilon_{FRET}$) from EGFP to alexa 594 (bound to anti-EGFP $V_HH$-$His_6$) [30]. In this case, $\varepsilon_{FRET}$ shows a broad distribution ranging from 0.2 to 0.9, and fluctuates with time. The time dependent fluctuation of the donor-acceptor distance, $R_{DA}$ (from 20 to 75 Å, Fig. 1A) clearly demonstrates that the structure of the antigen-antibody complex is not rigid and is actually quiet flexible [30]. This suggests that even in the case of specific binding of an antigen to its antibody, the structure is not rigid and involves significant local structural fluctuations.

## 3. Conformational dynamics of a DNA quadruplex

G-quadruplexes are an important class of DNA structure which is associated with the transcriptional regulation of various oncogenes, cancer therapeutics and DNA nanotechnologies [31–35]. Formation of G-quadruplex structure (Scheme 2) plays a crucial role in altering telomere length [36]. A telomere comprises of repetitive nucleotide sequences at each end of a chromosome and protects the chromosome from degradation or aggregation with neighbouring chromosomes which leads to DNA damage either way [37,38]. Telomerase is overexpressed in the cancer cells and maintains telomere length which promotes tumour progression [39,40]. Shortening of telomere length without altering telomerase level is a well-known strategy for tumour suppression [36]. There are some DNA binding proteins (hPOT1) which prevent the chromosome end aggregation and consequent DNA damage [37,38]. Formation of G-quadruplex structure involves competitive binding with hPOT1 and thus acts as a DNA damage signal in a cancer cell.

Addition of $K^+$ ion induces DNA quadruplex formation. Dash and co-workers recently demonstrated that a carbazole containing small molecule (BTC, Scheme 1C) alters the structural dynamics in a G-quadruplex DNA [31]. In order to carry out FCS and smFRET,